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Expression of tumor-associated glycoprotein-72 (TAG-72) antigen in human prostatic adenocarcinomas

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Abstract. Tumor-specific antigens are usually defined by monoclonal antibodies (MAbs) and can play critical roles in the diagnosis and therapy of carcinomas. Despite advances in the understanding of the molecular genetics of human prostate carcinomas, therapeutic approaches require that tumor-specific markers, preferably on the cell surface, should be defined. In this study, we examined the expression of an oncofetal antigen tumor-associated glycoprotein-72 (TAG-72) in prostatic adenocarcinomas with a Gleason grade of six or higher. Using a second generation MAb CC49 against TAG-72, immunoreactivity was detected in 88% (29/33) of the prostatic cancer tissues. Occasionally, the benign epithelium showed a very faint immunostaining but in most of the specimens, no reactivity was detected. Positive staining was present in the cytoplasm and the cell membrane of the malignant cells similar to reports on other cancer tissues. A weaker staining pattern of this antigen was seen in poorly differentiated areas. A significant negative correlation ($r=-0.36$, $p<0.05$) was observed between TAG-72 antigen expression and Gleason grade. The TAG-72 antigen expression in prostatic adenocarcinomas may be used as a target for radioimmunotherapy by the multivalent single chain antibody CC49 constructs recently generated by our group.

Introduction

Prostate cancer is the most common malignancy in the Western male populations. It is often an indolent disease but approximately 25-30% of the tumors behave aggressively resulting in approximately 40,000 deaths annually in the United States (1). In the majority of the aggressive cases, the tumor ultimately becomes androgen-independent. Patients with metastatic adenocarcinoma are frequently treated with chemical or surgical androgen ablation therapy. However, at the time of diagnosis, almost 33% of the patients have the advanced disease stage (2). Therefore, identification of tumor markers that are useful in the diagnosis and follow-up of patients with prostate cancer is a major task. Potential candidates, such as PSA (Prostate Specific Antigen), have not been successful in imaging trials, probably due to the fact that this antigen is a secreted protein (3). Some patients in whom conventional therapy has failed have been treated with radiolabeled CC49 against the tumor maker tumor-associated glycoprotein-72 (TAG-72) (4).

The TAG-72 is a high molecular weight glycoprotein related to the sialyted Tn antigen, and is frequently expressed in a variety of adenocarcinomas (5-10). Initially, the monoclonal antibody B72.3, prepared against a membrane-enriched extract of human breast carcinoma (11), was used to evaluate the expression of the TAG-72 antigen. It has been demonstrated that TAG-72 is not expressed in neural tumors or in adult normal tissues except in secretory endometrium; however, its expression was detected in certain fetal tissues (12,13). Thus, the fact that TAG-72 is positive in many adenocarcinomas while lacking a significant reactivity with normal tissues suggests the potential diagnostic and therapeutic utility for human carcinomas, and has been widely investigated in recent years (14,15).

Earlier studies of TAG-72 expression in prostatic adenocarcinomas have been limited to B72.3 that has a lower affinity than CC49 (16). Several other antibodies such as CC49 and CC83 have also been developed to detect the TAG-72 antigen (16). Each of these antibodies recognizes a specific epitope on the TAG-72 antigen. Radiolabeled CC49 monoclonal antibody (a second generation, high affinity antibody against TAG-72) has been used in several clinical trials. It showed the best tumor targeting in patients with breast, colon, and prostate cancer (4,17-19). The objective of the present study was to analyze the expression of TAG-72 antigen in a large series of moderate and high-grade human prostatic adenocarcinomas using CC49 monoclonal antibody.

Materials and methods

Prostatic adenocarcinoma tissues were obtained from 33 patients. The tissue was fixed in 10% neutral formalin, embedded in paraffin and 5 μm sections were stained with

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hematoxylin and eosin for pathological evaluation. The combined Gleason grade and pathological stage was assessed by one of the authors (S.L. Johansson) according to AJCC (20). Additional 5 μm thick tissue sections cut from the paraffin block were stained with the monoclonal antibody CC49 for TAG-72 expression by immunohistochemical (IHC) technique with the appropriate positive and negative controls.

The tumor tissue sections on the slide were deparaffinized using EZ-DeWax solution (BioGenex, CA). The slides were kept twice in a series of EZ-DeWax solution for 5-7 min, and then rinsed several times with distilled water. The tissue sections were washed three times with PBS (phosphate buffer saline). The tissue peroxidase activity was blocked by an autoblocker (Research Genetics, AL) for 5 min. Again the tissue samples were washed three times with PBS and incubated with the diluted Vectastain Horse Normal Serum (provided in the kit) in which the secondary antibody was raised, for 20 min to block the non-specific antigen-antibody immunoreactivity. The samples were incubated overnight at 4°C with 1:500 dilution of a stock solution of 2.6 mg/ml of primary antibody CC49. The IHC reaction was detected by using an ABC Elite Kit (Vector Laboratories, CA) as per the manufacturer's instructions. A reddish-brown precipitate indicated positive immunoreactivity.

The intensity of immunoreactivity of CC49 with TAG-72 antigen was scored independently by three investigators. Staining intensity was graded on 0-3 scale i.e., 0 for no staining, 1+ for week immunoreactivity; 2+ for intermediate immunoreactivity and 3+ for strong immunoreactivity. The extent of the staining was scored as follows: less than 25% of tumor cells stained (1); 25-50% of the tumor cells stained positive (2); 50-75% of the tumor cells stained positive (3) and more than 75% of the tumor cells stained positive (4). Intensity and extent of staining scores were multiplied with the maximum score being 12.

Results

We examined the expression of TAG-72 antigen in 33 specimens of the prostatic adenocarcinomas with CC49 monoclonal antibody by immunohistochemical analysis. Four of the 33 samples did not show any immunoreactivity for unknown reasons. In this study, 73% (24/33) of the specimens had Gleason grade 7 or higher while 27% had Gleason grade 6. Thus, the analyzed samples were moderately (Gleason grade 6) or moderately poorly differentiated (Gleason grade 7) or poorly differentiated (Gleason grade 8-10). Most of the patients had localized disease (stage T2), or an extra-capsular extension or an invasion of seminal vesicles (stage T3). Gleason grade, tumor stage and the extent of staining as well as a combined staining score are given in Table I.

Table I. TAG-72 expression in 33 specimens of prostatic adenocarcinomas with CC49 antibody by immunohistochemical analysis.

Specimen number	Gleasons grade	Stage	TAG-72 staining pattern	Extent of staining	Total staining score
1	6	T2	1	4	4
2	7	T2	2	2	4
3	7	T3	1	1	1
4	7	T2	1	1	1
5	6	T2	2	3	6
6	8	T2	1	1	1
7	8	T2	0	0	0
8	7	T2	2	2	4
9	6	T2	3	3	9
10	7	T2	1	2	2
11	7	T2	2	3	6
12	7	T3	2	1	2
13	7	T2	0	0	0
14	6	T2	2	1	2
15	7	T2	1	1	1
16	9	T3	0	0	0
17	6	T2	1	3	3
18	8	T2	1	3	3
19	7	T2	1	3	3
20	7	T2	1	2	2
21	8	T2	1	1	1
22	9	T3	1	1	1
23	6	T2	2	3	6
24	8	T2	2	1	3
25	6	T1	1	1	1
26	7	T3	2	1	2
27	8	T2	1	2	2
28	6	T2	2	1	2
29	9	T3	1	2	3
30	7	T2	3	4	12
31	6	T1	0	0	0
32	8	T3	1	2	2
33	9	T3	1	3	3

Independent of Gleason grade, <25% of the tumor cells exhibited immunoreactivity in 10 specimens, 25-50% in 8 specimens, 50-75% in 9 cases, while positive staining in >75% cell populations was observed in only 2 cases. A heterogeneous staining pattern was observed among the analyzed specimens of prostatic adenocarcinomas either based on the intensity of the staining pattern [coefficient of variation (CV)=45.5%] or the extent of the staining (CV=65%) as detailed in Table I. Furthermore, a remarkable heterogeneity was also observed for stage T2 (CV=86%) and stage T3 (CV=59%). The overall heterogeneity pattern within as well as between the stages was on the margin of the significant level. This lack of significance in the present study may be due to too few patients with stage T3 disease.

The localization and expression pattern of the TAG-72 antigen was very similar in all the specimens. Generally, most of the cases (87%) showed cytoplasmic immunoreactivity of the malignant cells (Fig. 1). Only three specimens revealed

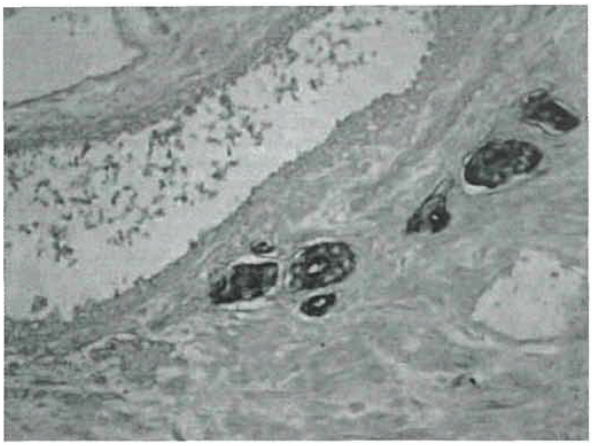

Figure 1. Prostatic adenocarcinoma, Gleason grade 7, showing a strong positive staining for TAG-72. Note the non-reactive benign gland adjacent to the malignant glands.

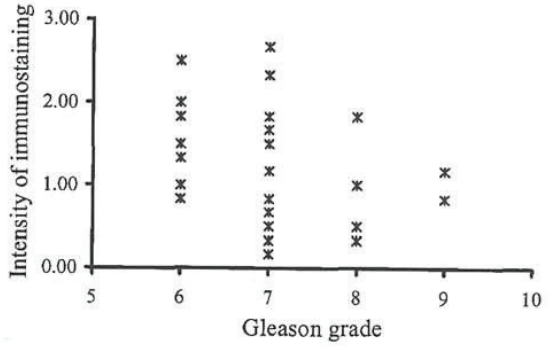

Figure 2. Immunostaining evaluation of TAG-72 antigen expression in prostatic adenocarcinomas.

membrane-bound staining, and two cases showed luminal staining of the malignant cells. The percentage of positively stained cells varied considerably among the specimens. On average, 25-70% of the tumor cells demonstrated a positive immunoreactivity with CC49 monoclonal antibody. The TAG-72 antigen expression was not detected in any of the stromal cells. However, only a few glandular cells (<25%) in the areas of benign prostatic hyperplasia (BPH) showed a very faint granular cytoplasmic staining, while 12 of the 33 specimens were completely negative for CC49 immunostaining. The areas of prostatic intraepithelial neoplasia (PIN) were seen in seven specimens of prostatic adenocarcinomas and showed staining intensities between 1 and 2. In these areas, the staining pattern was uniform and 5 cases showed immunoreactivity in 25-50% cells while 2 cases had 50-75% positive cells.

We evaluated a possible relationship between tumor grade and TAG-72 antigen expression. The correlation between the intensity of staining and the Gleason grade is shown in Fig. 2. Moderately differentiated tumors (Gleason grade 6 and 7) exhibited a higher level of immunoreactivity than poorly differentiated tumors (Gleason grade >7). There was a significant negative correlation ($r=-0.36$, $p<0.05$) of TAG-72 antigen expression with increasing Gleason grade.

Discussion

The present study aimed to assess the expression of a tumor-associated glycoprotein (TAG-72) in prostate cancer tissue samples. TAG-72 is a high molecular weight glycoprotein of the mucin family, which is expressed on a range of human carcinomas including colorectal, gastric, pancreatic, ovarian, endometrial, breast, non-small cell lung, and prostate (5-10). Altered expression of mucins has been associated with the histopathology of various tumors. TAG-72 may be aberrantly glycosylated in tumors, and hence becoming more attractive for immunotherapeutic studies (21,22). Monoclonal antibody CC49 is the prototype monoclonal antibody of such studies.

In the present study, we observed a heterogeneous immunostaining pattern with CC49 within the prostatic tumor specimens. When positive, the tumors generally showed a cytoplasmic distribution of TAG-72 expression similar to previously published results (8,10,23-25). Tumors that contain the areas of moderate differentiation (Gleason pattern 3) showed a higher tendency for TAG-72 antigen expression as compared to the adjacent poorly differentiated tumors (Gleason pattern 4 or 5). In contrast, in some specimens the moderately differentiated tumor areas also exhibited a very faint staining. Though it is difficult to explain, probably these cells may have lost their antigenicity due to differential glycosylation. We observed a decreased expression pattern of the TAG-72 antigen in poorly differentiated tumors, which may be due to the reduced secretory activity of such tumors. Previous studies also showed an inverse correlation of the prostate specific antigen with Gleason grade (26). It is not possible to compare the exact expression pattern of the TAG-72 antigen because of the lack of well-differentiated specimens and normal tissue samples. Furthermore, we could not correlate our data with the developmental stage since most of the analyzed tumors were stage T2 and T3.

In previous studies, a low expression level of the TAG-72 antigen was seen in BPH with B72.3 antibody (24,27). We also observed a very weak and faint staining in the benign epithelium of prostatic adenocarcinomas in 61% specimens, and in the areas of acute prostatitis. Moderately differentiated tumors (Gleason grade 3+3) had a stronger staining than the benign glandular epithelium. It does not necessarily mean that TAG-72 antigen is absent in the benign epithelium but it may be due to the limitations of the sensitivity of the immunohistochemical technique.

The monoclonal antibody CC49 has already entered clinical trials for radionuclide images and treatment of colon, breast, ovarian and prostate cancer (17-19). These studies have shown the potential of the CC49 monoclonal antibody in identifying the tumor antigen targets such as TAG-72. Recently, an improved approach for therapeutic application has been demonstrated by a genetically engineered tetravalent form of CC49 (28). The results indicate that this tetravalent form of CC49 may be more sensitive in recognizing the TAG-72 as a tumor marker in various types of carcinomas including prostatic adenocarcinomas.

In conclusion, this study demonstrates the expression of the TAG-72 antigen detected with the monoclonal antibody CC49 in malignant cells of prostatic adenocarcinomas, supporting previous studies that TAG-72 may be a potential target for prostatic adenocarcinomas with a CC49 antibody.

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